

Supporting Information

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SI Materials and Methods

miRNA Profiling Comparison of Lung Cancers from Never-Smokers and Smokers. Ohio State miRNA microarray data for the present 28 never-smoker cases (version 3.0) and for 58 smoker lung adenocarcinoma cases in our previous study (version 1.0) (1) and 23 additional smoker cases (version 2.0) (Table S2) were analyzed. Expression data comprising only the probes that were in common among all versions were LOESS normalized within each version group using statistical software R. Next, z-scores were calculated within each version, and data from all versions were merged. The merged dataset then was imported into BRB-ArrayTools version 3.5.0 to identify differentially expressed miRNAs ($P < 0.01$, FDR < 0.2).

mRNA Expression Data of Host Genes. Messenger RNA microarray data of 20 never-smoker lung adenocarcinoma cases were down-

loaded from the GEO database (2) (<http://www.ncbi.nlm.nih.gov/geo/>, GSE10072) and were analyzed by BRB-ArrayTools version 3.5.0.

Antibodies. The antibodies detecting EGFR, p-EGFR (Tyr-1173), p-Akt (Ser-473), PARP, and β -actin were purchased from Cell Signaling Technology.

Antisense Oligonucleotides. 2'-O-methyl oligonucleotides were synthesized at Integrated DNA Technologies. The sequence of antisense to miR-21 (anti-miR-21) was 5'-UCA ACA UCA GUC UGA UAA GCUA-3'. The sequence for enhanced green fluorescent protein (anti-EGFP as a control) was 5'-AAG GCA AGC UGA CCC UGA AGU-3'.

1. Yanaihara N, et al. (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 9:189–198.

2. Landi MT, et al. (2008) Gene expression signature of cigarette smoking and its role in lung adenocarcinoma development and survival. *PLoS ONE* 20:e1651.

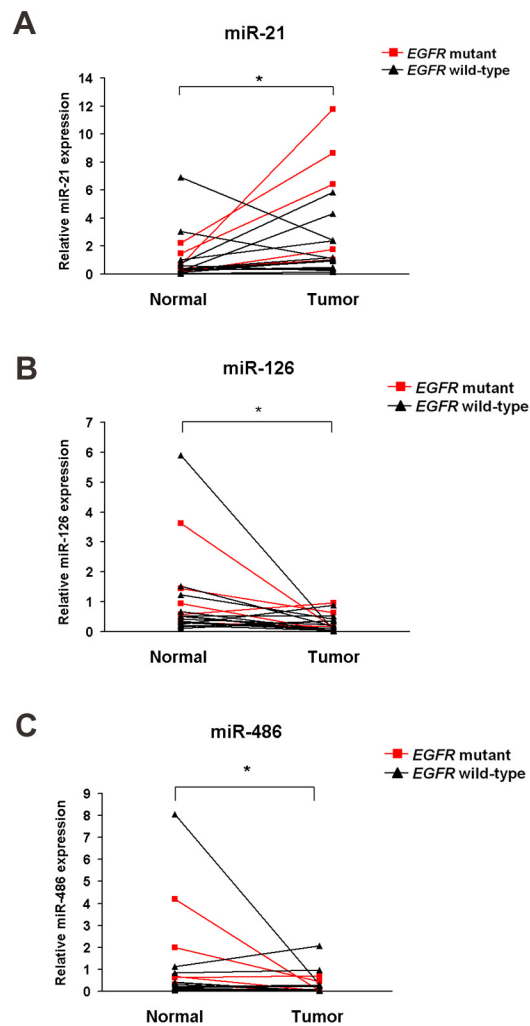


Fig. S1. MiR-21 (A), miR-126 (B), and miR-486 (C) expression from never-smoker samples. Expression levels of each miRNA in 20 pairs of tumor and normal tissues were analyzed using qRT-PCR. Of the 20 pairs, 15 cases were *EGFR* wild-type (cases no. 1, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 23, 26, and 27), and 5 cases were *EGFR* mutant (cases no. 2, 4, 24, 25, and 28). The 5 tumors expressing high levels of miR-21 were from 3 *EGFR*-mutant cases (cases no. 24, 25, and 28) and 2 *EGFR* wild-type cases (cases no. 5 and 23). Reactions were in triplicate for each sample. The expression levels were normalized with RNU6B, determined using the $2^{-\Delta\Delta CT}$ method and are presented relative to the mean value of normal tissue. *, $P < 0.05$, paired t -test.

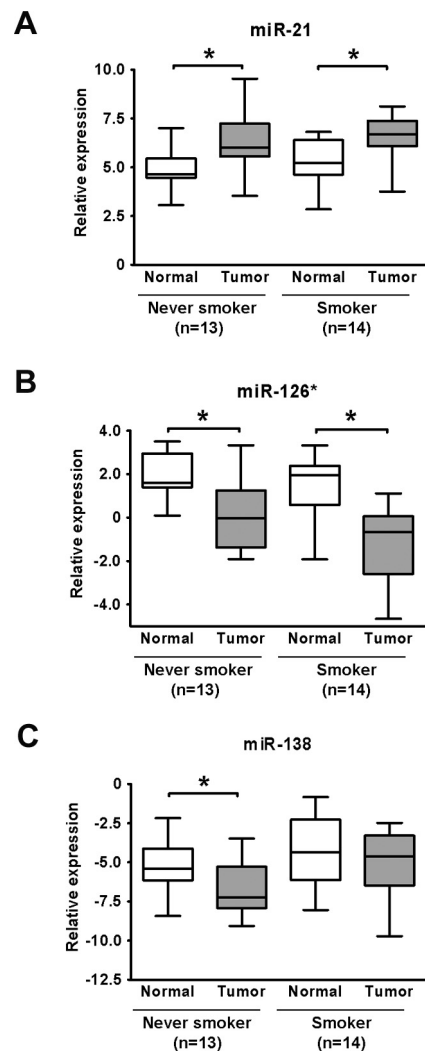


Fig. S2. MiR-21 (A), miR-126* (B), and miR-138 (C) expression in never-smokers versus smokers. By qRT-PCR, 13 pairs of lung adenocarcinoma and normal lung tissues from never-smokers and 14 pairs of lung adenocarcinoma and normal lung tissues from smokers were analyzed. Reactions were in triplicate for each sample. Relative expression was quantified as $\text{Log}_2 2^{-\Delta\text{CT}}$, where $\Delta\text{CT} = (\text{CT}_{\text{miRNA}} - \text{CT}_{\text{RNU6B}})$. *, $P < 0.05$, paired t -test.

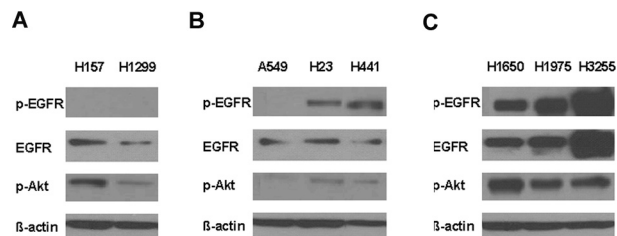


Fig. S3. Western blot analysis of 8 NSCLC cell lines. Protein expressions of *p*-EGFR, EGFR, and *p*-Akt were examined by Western blot analysis. (A) Non-adenocarcinoma cell lines (squamous cell carcinoma H157 and large cell carcinoma H1299). (B) Adenocarcinoma cell lines with wild-type *EGFR* (A549, H23, and H441). (C) Adenocarcinoma cell lines with mutant *EGFR* (H1650, H1975, and H3255). β -actin was a loading control. These images were quantified by measuring signal intensity using NIH Image J1.40g.

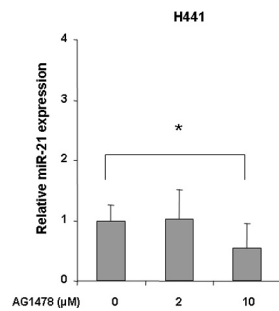


Fig. S4. AG1478 represses miR-21 expression in H441 lung adenocarcinoma cells. MiR-21 expression levels were analyzed by qRT-PCR after the AG1478 treatments (2 μM or 10 μM) in the absence of EGF and are expressed relative to untreated cells. Data are mean \pm SD from triplicate. *, $P < 0.05$, paired t-test.

Table S1. Characteristics of never-smoker patients with non-small cell lung cancer (n = 28)

Case no.	Age	Gender	Race	Histology	Stage	EGFR Status
1	68	M	Caucasian	Squamous cell carcinoma	I	Wild-type
2	48	F	Caucasian	Adenosquamous	II	ΔL747-S752 [†]
3	67	F	Caucasian	Adenocarcinoma	I	Wild-type
4	84	F	Caucasian	Adenocarcinoma	I	L858R [‡]
6	63	F	Caucasian	Adenocarcinoma	I	Wild-type
7	83	F	Caucasian	Adenocarcinoma	I	Wild-type
8	64	M	African American	Adenocarcinoma	I	Wild-type
9	61	F	Caucasian	Adenocarcinoma	I	Wild-type
10	65	M	Caucasian	Squamous cell carcinoma	I	Wild-type
11	68	M	Caucasian	Squamous cell carcinoma	III	Wild-type
12	84	M	Caucasian	Adenocarcinoma	I	Wild-type
13	65	M	Caucasian	Adenocarcinoma	IV	Wild-type
14	70	M	Caucasian	Unclassified	I	Wild-type
15	77	F	Caucasian	Adenocarcinoma	I	Wild-type
16	39	M	Caucasian	Adenocarcinoma	I	Wild-type
17	83	F	Caucasian	Adenocarcinoma	I	Wild-type
18	80	F	Caucasian	Adenocarcinoma	III	L858R [‡]
19	40	M	African American	Adenocarcinoma	III	Wild-type
20	36	F	African American	Adenocarcinoma	III	Wild-type
21	38	F	Caucasian	Adenocarcinoma	IV	Wild-type
22	70	F	Caucasian	Adenocarcinoma	I	Wild-type
23	49	F	Asian (Japanese)	Adenocarcinoma	I	Wild-type
24	66	F	Asian (Japanese)	Adenocarcinoma	I	L858R [‡]
25	67	F	Asian (Japanese)	Adenocarcinoma	I	L861E [§]
26	67	F	Asian (Japanese)	Adenocarcinoma	I	Wild-type
27	66	F	Asian (Japanese)	Adenocarcinoma	I	Wild-type
28	67	F	Asian (Japanese)	Adenocarcinoma	I	L858R [‡]

[†]In-frame deletion of codons 747 to 752.

[‡]Amino acid substitution from leucine to arginine at codon 858.

[§]Amino acid substitution from leucine to glutamate at codon 861.

Table S2. Characteristics of smoker patients with lung adenocarcinoma ($n = 23$)

Characteristic	No. of Patients
Stage	
I	14 (61%)
II	7 (30%)
III	2 (9%)
Age	
≤ 65	14 (61%)
> 65	9 (39%)
Gender	
Female	12 (52%)
Male	11 (48%)
Race	
Caucasian	15 (65%)
African	8 (35%)
American	

Table S3. Differentially expressed miRNAs related to smoking status

Mature miR	Probe	Location	P-Value [†]	FDR [‡]	Type [§]	Ratio	Cancer-Associated Genomic Changes	Host Gene ^{**}	Host Gene Association with Cancer
Common									
miR-21	hsa-mir-021-prec-17no1	17q23.1	0.008	0.184	Up	1.73	Amp: neuroblastoma; lung cancer	<i>TMEM49</i>	
			< 1e-07	< 1e-07		2.69			
miR-122	hsa-mir-122a-prec	18q21.31	0.001	0.083	Up	1.58	Del-bile duct cancer	Intergenic	
miR-126*	hsa-mir-123-precno1	9q34.3	0.005 0.001	0.022 0.083	Down	1.34 0.54	Del: NSCLC; HCC	<i>EGFL7</i>	Leukemia, colon cancer
			< 1e-07	< 1e-07		0.53			
miR-140	hsa-mir-140no2	16q22.1	0.005	0.158	Down	0.71		<i>WWP2</i>	
miR-30c	hsa-mir-030c-prec	6q13	0.001 0.005	0.008 0.158	Down	0.69 0.68	Del: lymphoma	Intergenic	
			0.002	0.012		0.75			
Never-smoker related									
miR-138	hsa-mir-138-1-prec	3p21.33	0.003	0.146	Down	0.58	Del: lung cancer, nasopharyngeal carcinoma	Intergenic	
Let-7c	hsa-let-7c-prec	21q21.1	0.009	0.184	Down	0.68		<i>AP000473.2 C21orf34</i>	Breast cancer
Smoker-related									
miR-210	hsa-mir-210-prec	11p15.5	< 1E-07	< 1E-07	Up	2.17	Del: ovarian; thyroid; kidney; bladder; breast; lung cancer	Intergenic	
miR-191	hsa-mir-191-prec	3p21.31	7.00E-07	1.32E-05	Up	1.58	Del: NSCLC; ovarian; head and neck squamous cell carcinoma; squamous cell cervical carcinoma	<i>DALRD3, C3orf60</i>	
miR-155	hsa-mir-155-prec	21q21.3	1.70E-06	2.57E-05	Up	1.6	Del: gastric cancer, amp-colon cancer	<i>BIC</i>	Lymphoma
miR-128	hsa-mir-128b-precno1	3p22.3	1.73E-05	2.18E-04	Up	1.56	Del: uterine cervical carcinoma; neuroblastoma; gallbladder cancer	<i>ARPP-21</i>	
miR-129	hsa-mir-129-precno2	11p11.2	2.27E-05	2.64E-04	Up	1.55	Del: prostate; breast; colon cancer	Intergenic	
miR-148a	hsa-mir-148-prec	7p15.2	4.23E-05	4.56E-04	Up	1.66	Del: squamous cell cervical carcinoma, amp-testicular germ cell tumors	Intergenic	
miR-7	hsa-mir-007-2-precno2	15q26.1	1.92E-04	0.002	Up	1.57	Amp: large cell lymphoma	Intergenic	
miR-17	hsa-mir-017-precno2	13q31.3	5.41E-04	0.004	Up	1.41	Amp: follicular lymphoma	<i>C13orf25, MIRHG1</i>	B-cell lymphoma, lung cancer
miR-102	hsa-mir-102-prec-1	1q32.2	6.09E-04	0.005	Up	1.36		intergenic	

Mature miR	Probe	Location	P-Value [†]	FDR [‡]	Type [§]	Ratio	Cancer-Associated Genomic Changes	Host Gene ^{**}	Host Gene Association with Cancer
miR-20a	hsa-mir-020-prec	13q31.3	0.001	0.007	Up	1.42	Amp: follicular lymphoma	<i>C13orf25</i> , <i>MIRHG1</i>	B-cell lymphoma, lung cancer
miR-146	hsa-mir-146-prec	5q33.3	0.002	0.01	Up	1.33		miRNA	
miR-200a	hsa-mir-200a-prec	1p36.33	0.002	0.012	Up	1.39	Del: colorectal; malignant pleural mesothelioma; lymphoma; Amp: ovarian; squamous cell cervical carcinoma	Intergenic	
miR-106	hsa-mir-106-prec-x	Xq26.2	0.002	0.012	Up	1.36	Del: ovarian cancer	Intergenic	T-cell lymphoma
miR-99b	hsa-mir-099b-prec-19no1	19q13.33	0.004	0.016	Up	1.41	Del: glioma	Intergenic	
miR-199a	hsa-mir-199a-1-prec	19p13.2	0.004	0.016	Up	1.29	Del: thyroid cancer, amp-large cell lymphoma	<i>DNM2</i>	
miR-9	hsa-mir-009-1no1	1q22	0.004	0.016	Up	1.32	Del: basal cell carcinoma	<i>C1orf61</i> , <i>AL139130.28</i>	
miR-214	hsa-mir-214-prec	1q24.3	0.008	0.03	Up	1.3	Del: T-cell lymphoma	<i>DNM3</i>	
miR-136	hsa-mir-136-precno2	14q32.31	0.008	0.03	Up	1.22	Del: nasopharyngeal cancer	Intergenic	
miR-24	hsa-mir-024-2-prec	19p13.12	0.01	0.035	Up	1.24	Amp: ovarian cancer	Intergenic	
miR-142	hsa-mir-142-prec	17q22	0.01	0.035	Up	1.36	t(8;17): Prolymphocytic leukemia	Intergenic	
miR-30a	hsa-mir-030a-precno1	6q13	< 1E-07	< 1E-07	Down	0.55	Del: lymphoma	<i>C6orf155</i>	
	hsa-mir-030a-precno2		1.04E-05	1.43E-04	Down	0.68		Intergenic	
miR-145	hsa-mir-145-prec	5q33.1	1.00E-07	2.50E-06	Down	0.6	Del: prostate cancer; myelodysplastic syndrome	Intergenic	
miR-30d	hsa-mir-030d-precno2	8q24.22	1.00E-07	2.50E-06	Down	0.59	Amp: pancreatic cancer	Intergenic	
miR-218-2	hsa-mir-218-2-precno2	5q35.1	2.00E-07	4.30E-06	Down	0.66	t(5;14)(q35.1;q32.2): Leukemia	Intergenic	
miR-9	hsa-mir-009-3no1	15q26.1	1.50E-06	2.52E-05	Down	0.71	Amp: large cell lymphoma	Intergenic	
miR-29b	hsa-mir-29b-2 = 102prec7.1 = 7.2	7q32.3	1.47E-04	0.001	Down	0.68	Del: prostate cancer	<i>AP4M1</i>	
miR-204	hsa-mir-204-precno2	9q21.11	5.18E-04	0.004	Down	0.71		<i>TRPM3</i>	
miR-30b	hsa-mir-030b-precno1	8q24.22	7.32E-04	0.005	Down	0.74	Amp: pancreatic cancer	Intergenic	
miR-125a	hsa-mir-125a-precno1	19q13.33	9.73E-04	0.007	Down	0.76	Del: glioma	Intergenic	
miR-224	hsa-mir-224-prec	Xq28	0.001	0.007	Down	0.77	Del: ovarian; squamous cell cervical carcinoma	<i>GABRE</i>	
miR-124	hsa-mir-124a-1-prec1	8p23.1	0.002	0.010	Down	0.72	Amp: malignant fibrous histiocytoma	Intergenic	
miR-96	hsa-mir-096-prec-7no2	7q32.2	0.003	0.014	Down	0.78	Del: prostate cancer	<i>AP4M1</i>	

Mature miR	Probe	Location	P-Value [†]	FDR [‡]	Type [§]	Ratio [¶]	Cancer-Associated Genomic Changes	Host Gene ^{**}	Host Gene Association with Cancer
miR-208	hsa-mir-208-prec	14q11.2	0.003	0.015	Down	0.75	Del: nasopharyngeal carcinoma; GIST, amp-NSCLC; hepatoblastoma	<i>MYH6</i>	
miR-193b	hsa-mir-193-precno2	16p13.12	0.004	0.019	Down	0.76		Intergenic	
miR-223	hsa-mir-223-prec	Xq12	0.007	0.029	Down	0.8	Del: ovarian; squamous cell cervical carcinoma, amp: prostate cancer	Intergenic	
miR-188	hsa-mir-188-prec	Xp11.23	0.008	0.030	Down	0.76	Amp: ovarian cancer	<i>CLCN5</i>	Renal cell carcinoma

Amp, amplification; del, deletion; HCC, hepatocellular carcinoma; GIST, gastrointestinal stromal tumor; miRNA, mRNA-like non-coding RNA; NSCLC, non-small cell lung cancer.

[†]MiRNA-microarray analysis was performed using pairs of tumors and corresponding normal tissues from 28 never-smokers and 81 smokers ($P < 0.01$).

[‡]False-discovery rate.

[§]Up, up-regulated in tumors compared with normal tissues; down, down-regulated in tumors compared with normal tissues.

[¶]Ratio of tumor to normal tissue.

^{||}Information was obtained from a previous report (1) and from the PubMed data base (<http://www.ncbi.nlm.nih.gov/sites/entrez>).

^{**}<http://microrna.sanger.ac.uk/sequences/>.

1. Calin GA, et al. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 101:2999–3004.